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STUDIES ON THE CHEMISTRY OF ANAPHYLAXIS (III).* EXPERIMENTS WITH ISOLATED PROTEINS, ESPECIALLY THOSE OF THE HEN'S EGG.†

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In the early history of immunology the few preliminary attempts to secure precipitins differentiating different proteins coming from the same animal were so generally unsuccessful that, in spite of some more or less positive results, the opinion became general that the specificity of proteins depends upon certain groups or radicals which are characteristic of the origin of the protein, and perhaps not associated with demonstrable chemical differences. With extension of the investigations to a more varied class of materials, and with refinements of the methods, the accuracy of this hypothesis became more and more questionable, until within the past few years it has come to be recognized that two different proteins from one and the same animal may, in certain cases, be distinguishable by means of the biological reactions. We now consider that a protein may exhibit a specificity characterizing its biological origin, which presumably depends upon certain hypothetical groups common to the animals of a particular origin, and that at least some proteins possess groups which are independent of their origin, and are in characteristic relation to the chemical composition of the proteins. On the one hand it has been shown that the precipitins for one protein of an animal may not react with another protein of the same animal, this having been demonstrated with milk proteins against serum proteins, serum proteins against hemoglobin, lens proteins against serum and tissue proteins. In these cases apparently common species groups are absent. On the other hand we find that antibodies for proteins of one species may react with corresponding proteins of other species; e.g., antibodies for lens proteins of one species will react with lens proteins of an entirely

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† The preceding articles of this series were published as follows: I, this *Journal*, 1908, 5, pp. 449–83; II, *ibid.*, 1909, 6, pp. 506–22.

different species, when they do not react with the serum or tissue proteins of homologous species. Antisera for casein are also said to react much more strongly with casein of foreign species than with lactalbumin or serum albumin of the same species (Bauer). Amyloid obtained from different species of animals reacts with antiserum obtained by immunizing with any sort of amyloid, but antiamyloid serum will not react with the serum of the animal from which the amyloid is derived (Raubitschek[1]). Here we seem to have proteins of similar chemical nature which do not contain the antigen groups characteristic of most of the other proteins of the same animal body.

There still remain, however, many unanswered questions concerning the possibility of distinguishing by different biological reactions proteins of a single animal or plant, even when these proteins are distinctly different from one another according to chemical criteria; that is, we are still far from having successfully correlated the biological and chemical characters of proteins. As most of the work along this line has been done by means of the precipitin reaction, and as the anaphylaxis reaction possesses certain differences, especially as to the possibility of extremely minute quantities of the proteins causing a reaction in animals, it seemed desirable to investigate this problem as far as feasible by means of the anaphylaxis reaction, and thus, if possible, throw upon the field a new light from a different angle. Review of the literature also reveals much confusion and contradiction because of the use of unsuitable methods of separation of proteins, whereby oftentimes mixtures were assumed to be pure proteins, and in other cases chemical modifications of the proteins were unwittingly produced during purification, with misleading results. It does not seem necessary to give here a complete résumé of all this literature, but attention will be directed chiefly to the work which has been done with egg proteins. These were used for most of the experiments to be recorded in this article, because they seemed to offer an especially favorable material. In the first place in egg white we have a protein, *ovomucoid*, which cannot be coagulated by heat and is not irreversibly coagulated by alcohol, and thus it can be separated readily and completely from the other proteins of the egg white; second, the existence of two different parts, the yolk and the white, provides variety of material from a common source; third, at least one of the proteins of egg white can be readily crystallized and recrystallized; and fourth, the egg proteins have received exceptionally thorough chemical investigation.

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[1] Raubitschek, *Verhandl. Deut. Path. Gesell.*, 1910, 14, p. 273.

It has long been recognized that egg white shows a less marked specificity than serum proteins, both as regards precipitin and anaphylaxis reactions. Thus in the early days of precipitin studies Myers[1] observed that precipitins resulting from immunizing with crystallized albumin from fresh hen's eggs reacted slightly with unpurified duck egg white, but not with purified albumin from sheep and bullock blood. Uhlenhuth[2] also found that antiserum for hen egg reacts with pigeon egg white, and conversely, but in each case the reaction was less marked than with the homologous egg white. Rosenau and Anderson[3] in one of their early papers on anaphylaxis reported a series of experiments on guinea-pigs with the whites of eggs of several species, which, as summarized below, exhibited a very irregular and uncertain individuality.

Sensitized	with	hen	egg	white	reacted	strongly	with	crane	egg	white
“	“	“	“	“	“	little	“	duck	“	“
“	“	duck	“	“	“	fatally	“	hen	“	“
“	“	guinea-hen	“	“	“	“	“	“	“	“
“	“	“ “	“	“	“	strongly	“	crane	“	“
“	“	pigeon	“	“	“	“	“	“	“	“
“	“	“	“	“	“	negatively	“	hen	“	“
“	“	goose	“	“	“	strongly	“	“	“	“
“	“	“	“	“	“	“	“	turkey	“	“
“	“	turkey	“	“	“	fatally	“	hen	“	“
“	“	“	“	“	“	strongly	“	crane	“	“

These and similar atypical and indefinite results are explained by the recent studies of Welsh and Chapman,[4] who, applying the saturation method to precipitating sera, demonstrate that antiserum for any egg white contains a general avian antisubstance, so that common precipitin reactions are obtained with all bird egg proteins, and also the antiserum contains an independent antisubstance specific for the species. Therefore by quantitative methods it can be found that a greater amount of precipitate is produced with the homologous antiserum, which reacts with both antigens, than with a heterologous antiserum which reacts only with the common avian antigen.

As to the relations of the different proteins which exist, or have been supposed to exist, in egg white, the literature contains but few reports. Levene[5] found that antiserum for whole egg white reacts to both albumin and globulin of egg white, as well as to yolk and chicken serum. Umber[6] found that antiglobulin serum and antialbumin serum both precipitate egg white; globulin as purified by him is precipitated by both these antisera, while crystallized albumin he found not precipitated by either. Obermeyer and Pick[1] divided the proteins of egg albumin into four portions (ovomucin, dysglobulin, euglobulin, and pseudoglobulin) and found that precipitins for each fraction reacted with all the other fractions, although irregularly and without distinct specificity.

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[1] *Lancet*, 1900 (ii), p. 98; *Centralbl. f. Bakt.*, 1908, 28, p. 237.

[2] *Deut. med. Wchnschr.*, 1900, 26, p. 734.

[3] *Bull. Hyg. Lab.*, No. 45.

[4] *Jour. of Hyg.*, 1910, 10, p. 177.

[5] *Medical News*, December 21, 1901.

[6] *Berl. klin. Wchnschr.*, 1902, 39, p. 657.

PREPARATION AND PROPERITIES OF MATERIAL USED.

The proteins from the egg white used in these experiments correspond in a way to those ordinarily designated as ovoglobulin, ovalbumin, and ovomucoid; that is, (1) the fraction precipitated on adding ammonium sulphate to half saturation (ovoglobulin); (2) the protein which crystallizes out on adding acetic acid to the half saturated solution (ovalbumin); and (3) the protein which is not coagulated by heat and which dissolves in water after being precipitated with alcohol (ovomucoid). In order to separate the albumin and "globulin" as completely as possible, the following procedure was followed: The precipitate obtained on beating up the whites of perfectly fresh eggs with an equal volume of ammonium sulphate solution was washed with half saturated ammonium sulphate solution, dissolved in water, and reprecipitated by adding an equal volume of saturated ammonium sulphate solution. After repeating this precipitation three times, three more precipitations were made by adding each time somewhat less than enough ammonium sulphate solution to render the concentration one-half saturation; the amount was determined by the degree of precipitation, and was roughly one-third to three-eighths saturation. In this way about one-half the protein in the original precipitate was eliminated and theoretically none of the ovalbumin should have been included with the "globulin" fraction.

To secure ovalbumin as free as possible from "globulin," the procedure was as follows: After crystallizing the ovalbumin three times in the usual manner, it was then precipitated three times with ammonium sulphate, which was added until a precipitate began to form, and this first part of the precipitate was discarded, the albumin in the filtrate being precipitated out with a little more ammonium sulphate. By this means about half the original crystalline protein was eliminated, and only the part less readily precipitated by ammonium sulphate, and presumably most free from globulin, was obtained. That is, we have secured proteins representing the extreme ends of what we may call the "precipitation spectrum" of egg white.

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[1] *Wien. klin. Rundschau*, 1902, 16, p. 277.

Ovomucoid was prepared in the usual way by pouring diluted and beaten egg white into boiling water acidulated with acetic acid, filtering off the coagulated protein, concentrating to a small bulk, precipitating with alcohol, redissolving in water, and reprecipitating the protein with alcohol three to five times. In all, five preparations of ovomucoid were made, which agreed with one another in giving a strong reaction with Millon's reagent, no reaction for tryptophane, a purplish biuret reaction, and containing much reducing carbohydrate which could be split off by boiling with dilute hydrochloric acid. They differed somewhat in solubility, some dissolving easily in water, some requiring a little alkali for quick solution.

The ovomucoid seems to be a definite compound, and the ovalbumin prepared as above specified should also be a pure protein, although it is not fully established that crystallized egg albumin is one rather than a mixture of proteins. As to the first or "globulin" fraction, there is much question, since the study of egg white by Osborne and Campbell.[1] These investigators separated and examined seventeen fractions of the proteins of egg white, and came to the conclusion that egg white contains four definite proteins: (1) Ovomucin, a glycoprotein constituting about seven per cent of the total protein, which has heretofore been regarded as a globulin; (2) ovalbumin, the easily crystallized component, which constitutes about 50 per cent of the proteins, and yields 2 to 2.5 per cent of carbohydrates on hydrolysis with HCl; (3) conalbumin, which is the non-crystallizable fraction obtained after removing the ovalbumin, and which differs from the ovalbumin also in having a different rotatory power and in coagulating at a slightly lower temperature; (4) ovomucoid, which is separated from ovalbumin by fractional precipitation only with great difficulty, but is readily separated by heat coagulation. According to these findings my "globulin" should consist of ovomucin with perhaps some ovalbumin admixed in indefinite proportions; my ovalbumin and ovomucoid should be quite pure preparations of these two proteins.

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[1] *Jour. Am. Chem. Soc.*, 1900, 22, p. 413.

Ovovitellin from the yolk was studied with a preparation obtained from Dr. T. B. Osborne, which represented a sample of this protein which had been quite thoroughly freed from lecithin by alcohol; this process had robbed it of much of its solubility so that only quite dilute solutions could be obtained in 0.1 per cent NaOH, the strongest alkaline solution which it is safe to use for intraperitoneal injections. This protein is a nucleo-albumin, which yields a pseudonuclein on digestion with pepsin.

ANAPHYLAXIS WITH OVOVITELLIN.

Ovovitellin was not found very satisfactory for anaphylaxis experiments, because of its slight solubility in 0.1 per cent NaOH, yet some results of significance were obtained (Table 1). As reported in the previous paper (II), ovovitellin has distinct specificity against the proteins from egg white. This is now again shown in a larger series of animals, as applying not only to crystallized egg albumin, but also to ovomucoid. There was no distinct reaction between the ovovitellin from the hen's egg and that from the egg of the turtle[1] (*Chelona midas*), but the very slight solubility of the latter renders these results something less than conclusive (Table 1, experiments 19–22 and 32–39).

Crude egg white, obtained from fresh eggs, seems to contain either a trace of vitellin, or else something which sensitizes to vitellin, for experiments 1 to 3 show that crude egg white renders guinea-pigs sensitive to ovovitellin, but purified egg albumin does not sensitize to vitellin (experiments 4–9). Ovomucoid and vitellin are also found not to react with one another (experiments 10–13). Although ovovitellin does not cause strong anaphylactic reactions, yet the results in these experiments are distinct enough to demonstrate that this typical egg yolk protein is biologically distinct from two typical proteins of the white, namely, albumin and ovomucoid.

So far as I can find in the literature, purified yolk protein has not previously been studied as to any of its biological reactions.

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[1] For this preparation I am indebted to Professor L. B. Mendel.

TABLE I.
OVOVITELLIN.

	Sensitizing Dose	Days' Interval	Second Injection	Results	Subsequent Injections and Remarks
1......	0.001 gm. egg albumin, crude	24	0.08 gm. ovovitellin (hen)	Severe symptoms	48 hrs. later reacted fatally to egg albumin
2......	0.001 “ “ “ “	24	0.08 “ “ “	Moderate “	48 “ “ “ “ “ “ “
3......	0.001 “ “ “ “	24	0.08 “ “ “	“ “	48 “ “ “ “ “ “ “
4......	0.01 “ “ “ pure	20	0.05 “ “ “	No “	48 “ “ “ moderately to egg albumin
5......	0.002 “ “ “ “	20	0.05 “ “ “	“ “	48 “ “ “ “ “ “ “
6......	0.001 “ “ “ “	20	0.05 “ “ “	“ “	48 “ “ “ “ “ “ “
7......	0.0002 “ “ “ “	20	0.05 “ “ “	“ “	48 “ “ “ “ “ “ “
8......	0.01 “ “ “ “	22	0.05 “ “ “	“ “	
9......	0.002 “ “ “ “	22	0.05 “ “ “	“ “	
10......	0.01 “ ovomucoid A	20	0.05 “ “ “	Doubtful “	24 “ “ “ severely to ovomucoid
11......	0.002 “ “ “	20	0.05 “ “ “	No “	24 “ “ “ slightly “ “
12......	0.001 “ “ “	20	0.05 “ “ “	Doubtful “	24 “ “ “ “ “ “
13......	0.0002 “ “ “	20	0.05 “ “ “	No “	24 “ “ “ “ “ “
14......	0.001 “ egg globulin	31	0.05 “ “ “	Slight “	
15......	0.006 “ ovovitellin (hen)	20	0.075 “ egg albumin, pure	Doubtful “	24 “ “ “ “ “ ovovitellin (hen)
16......	0.002 “ “ “	20	0.075 “ “ “ “	“ “	
17......	0.001 “ “ “	20	0.075 “ “ “ “	“ “	
18......	0.0002 “ “ “	20	0.075 “ “ “ “	“ “	24 “ “ “ “ “ “ “
19......	0.006 “ “ “	20	ovovitellin (turtle)	No “	24 “ “ “ severely “ “ “
20......	0.002 “ “ “	20	“ “	“ “	
21......	0.001 “ “ “	20	“ “	“ “	24 “ “ “ moderately to ovovitellin (hen)
22......	0.0002 “ “ “	20	“ “	“ “	
23......	0.01 “ “ “	28	0.1 “ ovomucoid A	“ “	24 “ “ “ slightly “ “ “
24......	0.002 “ “ “	28	0.1 “ “ “	“ “	24 “ “ “ “ “ “ “
25......	0.001 “ “ “	28	0.1 “ “ “	“ “	24 “ “ “ “ “ “ “
26......	0.005 “ “ “	20	0.1 “ “ “	Doubtful “	24 “ “ “ severely “ “ “
27......	0.001 “ “ “	20	0.1 “ “ “	“ “	
28......	0.002 “ “ “	20	0.1 “ “ “	“ “	24 “ “ “ moderately “ “ “
29......	0.0001 “ “ “	20	0.1 “ “ “	“ “	24 “ “ “ severely “ “ “
30......	0.005 “ “ “	19	0.1 “ “ “	No “	48 “ “ “ slightly “ “ “
31......	0.001 “ “ “	19	0.1 “ “ “	“ “	48 “ “ “ moderately “ “ “
32......	0.01 “ “ (turtle)	21	ovovitellin (hen)	Slight “	24 “ “ “ slightly “ “ (turtle)
33......	0.002 “ “ “	21	“ “	Doubtful “	24 “ “ “ “ “ “ “
34......	0.001 “ “ “	21	“ “	Slight “	24 “ “ “ “ “ “ “
35......	0.0002 “ “ “	21	“ “	Doubtful “	24 “ “ “ “ “ “ “
36......	0.010 “ “ “	20	“ “	No “	
37......	0.002 “ “ “	20	“ “	“ “	
38......	0.001 “ “ “	20	“ “	“ “	
39......	0.0002 “ “ “	20	“ “	“ “	

There are a few statements as to precipitins for egg white and egg yolk, but these were made in the early days of immunological study, and apparently there is no evidence of an accurate separation of the two materials, against which separation there are serious mechanical obstacles.

EXPERIMENTS WITH OVOMUCOID.

The five preparations of ovomucoid studied differed somewhat in solubility, and also somewhat in toxicity for sensitized animals, in which respect they were all less active than globulin or albumin, but more active than the vitellin. Their relative toxicity, when given in doses of 0.1 to 0.5 gm. to guinea-pigs which had been sensitized with the homologous ovomucoid in doses from 0.010 to 0.0002 gm., is shown in the following table:

ANAPHYLACTIC ACTIVITY OF OVOMUCOID PREPARATIONS.

Preparation Used	Degree of Reaction					
	None or Doubtful	Slight	Moderate	Severe	Fatal	Total
A	2	7	1	4	0	14
B	3	0	3	0	0	6
C	0	0	2	1	1	4
D	0	1	1	1	2	5
E	0	1	1	3	1	6
Totals	5	9	8	9	4	35

In other experiments it was found that any ovomucoid preparation sensitized against the others as well as against itself. With all five specimens a definite specificity against crystallized egg white was obtained (Table 2), showing not only a distinct chemical specificity for each protein, but also that by repeated crystallization of egg albumin it can be made free from any appreciable amount of ovomucoid. A smaller number of experiments indicated the individuality of ovomucoid as against "globulin" of egg white, as well as against the vitellin of the yolk. In all cases injection of the heterologous protein did not reduce the sensitivity to the homologous protein to any noticeable degree, as shown by positive reactions when the homologous protein was injected after the heterologous.

TABLE 2.
OVOMUCOID.

	Sensitizing Dose	Days' Interval	Second Injection	Results	Subsequent Injections and Remarks
1	.005 gm. ovomucoid A	24	0.1 gm. albumin, cryst.	No symptoms	24 hrs. later reacted severely to ovomucoid A
2	.0005 " " A	24	0.1 " " "	" "	
3	.010 " " A	30	0.1 " " "	" "	24 " " " " " " A
4	.005 " " A	30	0.1 " " "	" "	
5	.001 " " A	30	0.1 " " "	" "	
6	.010 " " B	..	0.1 " " "		
7	.002 " " B	..	0.1 " " "		
8	.001 " " B	..	0.1 " " "		
9	.0002 " " B	..	0.1 " " "		
10	.002 " " C	20	0.1 " " "	No symptoms	
11	.001 " " C	20	0.1 " " "	" "	
12	.002 " " C	20	0.1 " " "	" "	
13	.002 " " C	20	0.1 " " "	Doubtful "	24 hrs. later reacted severely to ovomucoid C
14	.001 " " C	20	0.1 " " "	No "	24 " " " moderately " " C
15	.001 " " C	20	0.1 " " "	" "	
16	.010 " " D	20	0.1 " " "	Doubtful "	7 days " " fatally " " D
17	.002 " " D	20	0.1 " " "	" "	7 " " " " " " D
18	.005 " " E	19	0.1 " " "	" "	
19	.012 " " E	22	0.15 " " "	Slight "	24 hrs. " " moderately " " E
20	.010 " " E	16	0.1 " " "	No "	
21	.002 " " E	16	0.1 " " "	" "	
22	.001 " " E	16	0.1 " " "	" "	
23	.001 " " A	18	0.1 " globulin	" "	
24	.010 " " A	20	0.05 " ovovitellin (hen)	Slight "	24 " " " severely " " A
25	.002 " " A	20	0.05 " " "	No "	24 " " " slightly " " A
26	.001 " " A	20	0.05 " " "	Doubtful "	24 " " " " " " A
27	.0002 " " A	20	0.05 " " "	No "	24 " " " " " " A
28	.001 " egg albumin, cryst.	22	0.1 " ovomucoid A	" "	24 " " " fatally " albumin
29	.001 " " " "	22	0.1 " " A	" "	24 " " " " " "
30	.001 " " " "	28	0.1 " " A	" "	24 " " " " " "
31	.001 " " " "	28	0.1 " " A	Doubtful "	
32	.001 " " " "	28	0.1 " " A	No "	
33	.001 " " " "	22	0.1 " " B	Doubtful "	24 " " " " " "
34	.0001 " " " "	22	0.1 " " B	" "	24 " " " " " "
35	.001 " " " "	24	0.1 " " C	" "	24 " " " " " "
36	.001 " " " "	24	0.1 " " C	No "	
37	.010 " " " "	20	0.1 " " D	" "	
38	.002 " " " "	20	0.1 " " D	" "	
39	.001 " " " "	30	0.2 " " E	" "	
40	.002 " " " "	24	0.2 " " E	" "	24 " " " " " "
41	.001 " " globulin	31	0.1 " " A	" "	24 " " " severely " egg white
42	.001 " " "	31	0.1 " " B	" "	
43	.005 " ovovitellin	20	0.1 " " A	Doubtful "	24 " " " " " ovovitellin
44	.001 " "	20	0.1 " " A	" "	
45	.0005 " "	20	0.1 " " A	" "	24 " " " " " "
46	.0001 " "	20	0.1 " " A	" "	24 " " " moderately " "
47	.002 " "	19	0.1 " " A	No "	48 " " " slightly " "
48	.001 " "	19	0.1 " " A	" "	48 " " " moderately " "

EXPERIMENTS WITH THE "GLOBULIN" FRACTION OF EGG WHITE.

The published evidence as to the anaphylactic activity of the various proteins of serum is altogether contradictory. In studies of anaphylaxis with different fractions of serum, Gay and Adler[1] found that the euglobulin fraction (one-third saturation with ammonium sulphate) sensitizes as well as the entire serum, but when purified is not toxic, while the later fractions of serum proteins are highly toxic but less active in sensitizing. Cabannes,[2] on the other hand, found that the globulins are somewhat more toxic than the serum albumin. Bruynoghe,[3] however, found albumin, euglobulin, and pseudoglobulin all capable of sensitizing and intoxicating, the pseudoglobulin producing the least severe intoxication; while Doerr and Russ[4] state that the globulin alone exhibits either sensitizing or intoxicating properties. Doerr and Russ[5] also found that passive sensitization occurred only with the blood of rabbits immunized with globulin, and not when the immunization was performed with serum albumin.

There seems to be very little difference in activity between the fractions of egg white which correspond in precipitability with the albumin and globulin of serum.

It can be seen from experiments 1–11, Table 3, that the minimum fatal sensitizing dose of the egg "globulin" is very small, 0.000,001 gm. being efficient; fatal intoxication was obtained with 0.001 gm., while 0.0001 gm. caused a slight reaction. These figures are practically identical with those obtained with crystallized albumin, but it should be said that in general minimum doses of "globulin" produced fatal results somewhat less constantly than did corresponding doses of albumin. Therefore, in egg white the two chief proteins are almost equal in their degrees of anaphylactic toxicity, the albumin being slightly the more active of the two.

Experiments 12–26 show that the albumin and globulin fractions react with one another to a very considerable extent. Even very minute doses of one will sensitize a guinea-pig to the other protein, suggesting that these fractions may represent identical proteins.

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[1] *Jour. Med. Res.*, 1908, 18, p. 433.

[2] *Compt. Rend. Soc. Biol.*, 1907, 62, p. 809.

[3] *Arch. Internat. Pharmacodynam.*, 1909, 19, p. 393.

[4] *Zeit. Immunität*, 1909, 2, p. 109.

[5] *Ibid.*, 1909, 3, p. 181.

TABLE 3
"GLOBULIN" FRACTION OF EGG WHITE.

	Sensitizing Dose	Days' Interval	Second Injection	Results	Subsequent Injections and Remarks
1	.0001 gm. egg globulin	26	0.1 gm. egg globulin	Death, 16 min.	
2	.0001 " " "	26	0.1 " " "	" 25 "	
3	.00001 " " "	26	0.1 " " "	" 2 "	
4	.000001 " " "	26	0.1 " " "	" 45 "	
5	.0000001 " " "	26	0.1 " " "	Slight	
6	.0001 " " "	20	0.002 " " "	Death, 30 min.	
7	.0001 " " "	20	0.002 " " "	" 5 "	
8	.0001 " " "	30	0.001 " " "	" 30 "	
9	.001 " " "	30	0.0005 " " "	Moderate	3 days later reacted slightly to egg globulin
10	.0005 " " "	30	0.0005 " " "	Slight	3 " " " fatally " " "
11	.001 " " "	30	0.0001 " " "	"	3 " " " moderately " " "
12	.010 " " "	28	0.1 " albumin	Death, 20 min.	
13	.0001 " " "	28	0.1 " "	" 15 "	
14	.00005 " " "	28	0.1 " "	" 15 "	
15	.0005 " " "	21	0.001 " "	Severe	24 hrs. " " " " " "
16	.0005 " " "	22	0.002 " "	Slight	
17	.001 " " "	24	0.10 " "	"	
18	.001 " " "	31	0.10 " "	Death, 10 min.	
19	.001 " " "	31	0.10 " egg white	" 30 "	
20	.010 " albumin	28	0.1 " globulin	Moderate	24 " " " fatally " albumin
21	.0005 " "	28	0.1 " "	"	24 " " " " " "
22	.0001 " "	28	0.1 " "	"	24 " " " severely " "
23	.00001 " "	28	0.1 " "	"	24 " " " moderately " "
24	.001 " "	21	0.001 " "	No symptoms	24 " " " severely " "
25	.001 " "	22	0.001 " "	Slight	48 " " " fatally " "
26	.001 " "	24	0.002 " "	Moderate	24 " " " " " "

Against this assumption, however, is the fact that small intoxicating doses (1–2 milligrams) do not usually cause severe reactions with the heterologous sensitization. As the polysensitization experiments (described below) show, there really are two distinct proteins present in these two fractions, but evidently there is either a common protein mixed in both, or else there is a common antigen group in addition to the specific one. Possibly we have three proteins in the two fractions, in the "globulin" fraction being ovomucin and ovalbumin, in the crystallized albumin being ovalbumin and conalbumin or some other, unidentified protein. It is certainly an interesting coincidence, at least, that by the anaphylaxis reaction we find three antigens present in the coagulable part of egg white, and that chemical examination led Osborne and Campbell to distinguish three proteins in the same material.

In studying closely related or mixed proteins by the precipitin test the method of saturation has been found of great value. By adding one protein to an antiserum until all the specific precipitins have been exhausted, the presence of co-existing precipitins specific for a closely related protein then may be demonstrated by adding this protein to the exhausted antiserum. In this way it has been found possible to differentiate between sera of such closely related species as man and monkey, dog and fox, etc. The same principle has also been applied successfully with agglutinins and complement fixators, but, so far as I can learn, not in anaphylaxis. It is equally feasible in anaphylaxis, however, as the experiments in Table 4 demonstrate. If a sensitized animal is given a sufficient quantity of the specific antigen and recovers from the reaction, it will be refractory to another dose of the same antigen given within the next few days. According to Friedberger's hypothesis this phenomenon depends simply upon the exhaustion or saturation of the specific antibody, in favor of which is the fact which I have observed many times, that if the intoxicating dose is very small (1–2 milligrams of egg albumin) even although the animal reacts severely yet it will not usually be rendered refractory by this reaction, presumably because the amount of antigen injected has not combined all the antibody. Evidently it is not the severity of the reaction which determines

TABLE 4.

REACTION OF GUINEA-PIGS SENSITIZED TO WHOLE EGG WHITE (0.006 GM.) THREE WEEKS, TO ISOLATED EGG PROTEINS INJECTED AT 24 HOUR INTERVALS.

Second Injection	Result	Third Injection	Result	Fourth Injection	Result	Fifth Injection	Result	Sixth Injection	Result
Gm.		Gm.		Gm.		Gm.		Gm.	
1. .0.050 globulin	Severe	0.050 globulin	Slight	0.1 albumin	Died in 55 min.				
2. .0.050 "	"	0.050 "	None	0.1 "	" " 50 "				
3. .0.033 "	"	0.050 "	Slight	0.050 "	Moderate				
4. .0.033 "	"	0.050 "	"	0.050 "	"	0.1 whole egg white	Severe		
5. .0.033 "	"	0.050 "	"	0.050 "	Severe				
6. .0.002 albumin	Slight	0.010 albumin	"	0.050 "	Moderate	0.033 globulin	Severe	0.1 whole egg white	Moderate
7. .0.002 "	"	0.010 "	"	0.050 "	Doubtful	0.033 "	Moderate	0.1 " " "	Severe
8. .0.001 "	Moderate	0.0025 "	Moderate	0.050 "	"	0.050 "	"		
9. .0.001 "	"	0.0025 "	Doubtful	0.050 "	"	0.050 "	"		
10. .0.001 "	Severe	0.0025 "	"	0.050 "	Slight	0.050 "	"		
11. .0.001 "	"	0.0025 "	"	0.050 "	"	0.050 "	"		
12. .0.050 ovomucoid A	Slight	0.050 ovomucoid A	"	0.050 "	Died in 25 min.				
13. .0.050 " A	"	0.050 " A	"	0.050 "	" " 25 "				
14. .0.050 " A	"	0.050 " A	"	0.050 "	" " 10 "				
15. .0.010 " C	Moderate	0.040 " C	None	0.050 "	" " 30 "				
16. .0.010 " C	"	0.040 " C	"	0.050 "	Severe	0.050 "	Severe		
17. .0.010 " C	"	0.040 " C	"	0.050 "	"	0.050 "	None		
18. .0.050 vitellin	Doubtful	0.003 albumin	Severe	0.050 "	None	0.050 "	Severe		
19. .0.050 "	"	0.033 globulin	"	0.1 whole egg white	Severe				
20. .0.050 "	"	0.033 "	Slight	0.1 " " "	"				

the refractory condition, but the amount of injected antigen. Of similar import is the well known phenomenon of polysensitization as demonstrated by Rosenau and Anderson, that a guinea-pig sensitized with several proteins is specifically sensitive to all, and will react with any one after recovering from anaphylactic shock induced by any one of the others.

Taking advantage of these features of the anaphylaxis reaction it is possible to exhaust a specific anaphylactin in the body of a guinea-pig, leaving the animal still sensitized to other proteins. In this way the different proteins of egg white have been studied as to their identity. Guinea-pigs were sensitized with entire egg white from fresh eggs (given in doses containing 0.006 gm. of protein), which, of course, contains all the different distinct proteins which there may be in egg white. Pigs sensitized in this way will react, after the usual incubation period, to any of our three fractions of egg white ("globulin," albumin, and ovomucoid) but only doubtfully to vitellin from the yolk. If the sensitized animal is given two or more injections of one of these fractions at 24 hour intervals, rendering it entirely refractory to this particular protein, it will be found to be still sensitive to either one of the other fractions or to the entire egg white. This demonstrates conclusively that there are several proteins or antigens present in the egg white, which are biologically distinct from one another, although derived from the same species.

By this means it can be shown that guinea-pigs sensitized to whole egg white and made refractory to "globulin" will still react strongly or fatally to crystallized albumin, or if made refractory to albumin they will still react to globulin. This proves that there are proteins or at least antigens present in each of these fractions which are not present in the other fraction. It also proves at the same time that even the extreme method of separation by fractional precipitation with ammonium sulphate which was used in making these preparations is not adequate to separate the proteins of egg white, since at least one common protein or antigen is present in each fraction. From the standpoint of chemistry nothing else could be expected, but it seems desirable to demonstrate and emphasize again the inefficiency of the salting-out method as a means of separating proteins for biological study, in view of the persistent attempts which are made to do so. While the present work concerns only the proteins of egg white, there is no reason for believing that any more accurate fractionation can be obtained with serum or other natural mixtures of closely related proteins.

HYPERSENSITIZATION AND IMMUNITY BY FEEDING.

In a previous publication[1] evidence was cited which seemed to indicate that the feeding of a protein for a limited time renders a guinea-pig hypersensitive to the same protein, yet if the feeding was continued long enough the guinea-pig eventually becomes either immune or refractory. The experimental data at that time were limited, but further observations on this feature of anaphylaxis have given further support to the conclusions drawn.

The following observations have been made:

1. Guinea-pigs bred from mothers fed with oats were, as soon as weaned, put upon a diet of egg albumin and carrots. Other young pigs from the same stock were raised upon oats and carrots. The latter animals after reaching a weight of 250 to 300 gms. did not give anaphylactic reactions when injected with 0.05 gm. of a protein obtained from raw oats, and if given small doses, such as ordinarily given for sensitizing, they were not rendered sensitive to subsequent injections of 0.05 gm. of oat protein. Some of the pigs which were raised to 200 to 250 gms. weight without oats were found to give a typical reaction of moderate severity when injected once with 0.05 gm. oat protein, apparently from passive sensitization conferred by the mother. Others gave no reaction. After the animals fed without oats were somewhat older, 350 to 400 gms., they reacted much less strongly or not at all to oat protein, as if this inherited passive sensitization were passing off, as passive sensitization normally does; such pigs, if given sensitizing doses of oat protein, are found to be sensitive to this protein three weeks later, giving well defined reactions of moderate severity. Hence the conclusion seems warranted that if guinea-pigs are raised on oat proteins they cannot be made to give anaphylactic reactions with oat proteins, but if raised without oats they may be sensitized

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[1] Wells and Osborne, *Jour. Infect. Dis.*, 1911, 8, p. 77.

to oat proteins, just as they can be to other proteins not usually in their food. These experiments support the experience obtained previously with zein, that guinea-pigs become immune to the chief vegetable proteins of their food.

2. Guinea-pigs raised from the time of weaning on a diet of egg protein (Merck's dried egg albumin) and carrots were found, as in previous experiments, to give strong anaphylaxis reactions when injected with egg albumin between the thirtieth and sixtieth days, but later they reacted less strongly, and after the one hundredth day of feeding they gave but slight reactions to 0.1 gm. dried egg albumin. At this time sensitization with egg albumin can be obtained to only a slight degree, such guinea-pigs given injections of egg albumin showing but slight reaction to a subsequent dose of egg albumin, while control pigs fed on oats and carrots gave severe, usually fatal, reactions to corresponding injections of egg albumin. Apparently, then, daily absorption of animal protein in the food at first renders guinea-pigs hypersensitive to this protein, but if the feeding is kept up for a long enough period the animals become refractory to the food protein.

3. A series of guinea-pigs which were raised on bread and cow milk for 10 weeks were found at the end of this time to be still highly sensitive to milk, dying promptly when given 1 to 3 c.c. of milk intraperitoneally. Apparently this length of feeding is not sufficient to render guinea-pigs immune to milk. These results are not at all in harmony with those of Besredka,[1] who did not succeed in sensitizing with milk by either oral or rectal administration, but did find that sensitized guinea-pigs were made refractory to intracerebral injections of milk if previously given milk by either of these routes.

DOES EGG WHITE CONTAIN A SUBSTANCE INHIBITING THE ANAPHYLAXIS REACTION?

In a set of experiments previously reported (I) it was found that the minimum amount of crude egg white required to produce sensitization or intoxication was much larger than would be expected from its known content of crystallizable egg albumin, to say nothing of the other proteins. Thus, in one experiment the minimum amount of protein in the form of crude egg white which sensitized guinea-pigs was one hundred times greater than the minimum sensitizing dose of crystallized egg albumin, and the minimum lethal dose was five times greater. As the crystallizable albumin constitutes over half of the total egg proteins, this difference in reaction cannot be ascribed to the fact that only part of the egg protein is albumin, and it was suggested that raw egg proteins might possibly contain some substance inhibiting the anaphylaxis reaction, at least to some extent.[1] A repetition of this experiment with other samples of crude egg white gave similar results, as shown in the following table:

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[1] *Ann. Inst. Pasteur.*, 1909, 23, p. 166.

COMPARATIVE ACTIVITY OF CRUDE AND PURE EGG PROTEINS.

Protein Used	Minimum Sensitizing Dose Causing Slight Reaction	Minimum Sensitizing Dose Causing Fatal Reaction	Minimum Fatal Intoxicating Dose
Crystallized albumin	0.000,000,05	0.000,001	0.000,5
Purified globulin	0.000,000,1	0.000,001	0.000,8
Crude egg white protein (I)	0.000,003	0.000,012	0.001
Crude egg white protein (II)	0.000,006	0.000,5	0.002,5

One possibility was that the different proteins interfere with one another in the reaction. To test this, experiments were performed in which animals were sensitized with one of the proteins of the egg white and then allowed to react with two different proteins; but in a long series of experiments no definite evidence of inhibition could be obtained in this way.

As it appeared from these experiments that the inhibition was not caused by any of the proteins, the effect of the non-protein material was then tried, with but little more positive results. It was found that when a few milligrams of the non-coagulable material, obtained by evaporating to dryness the filtrate from the coagulated whites of several eggs, were added to crystallized albumin, the amount necessary to produce fatal sensitization was apparently somewhat increased; but there was no definite interference with the toxicity of crystallized albumin or pure globulin for sensitized animals. In a few experiments minimum lethal doses of albumin mixed with the residue failed to kill sensitized pigs, but in so many other cases this effect was not observed that the positive results cannot be considered as significant. This non-coagulable material, of course, includes ovomucoid, but as this protein was found not to affect the reaction, the slight results obtained must be ascribed to the non-protein constituents. Of these, the salts and the lipoids must be considered, and as yet we have not been able to investigate the responsibility of each of these groups of substances. Banzhaf and Steinhardt[1] found that egg lecithin affords no protection to an animal sensitized with serum if injected together with the second dose of serum, but if given intraperitoneally several hours previous to the second injection it protects quite effectively.

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[1] Cabannes (*Compt. Rend. Soc. Biol.*, 1907, 62, p. 809) also states that the proteins precipitated by ammonium sulphate are more toxic than the totality of the protein of serum.

As the reactivity of egg globulin seems to be if anything helped rather than impaired by the non-coagulable residue, it may be thought that the salts are concerned, since small amounts of salt favor the solution of globulin, and also since it is known that large amounts of sodium chloride interfere with the union of amboceptor and complement, thus inhibiting the anaphylaxis reaction. But taken all together the results of my experiments are too inconclusive to locate any true inhibiting substance which may exist in the egg white. There still remains the possibility that a thermolabile substance is present in egg white and not present in either the purified albumin or globulin, but its existence has not been established by my experiments. Fein,[2] however, obtained results pointing to such a possibility, for he found that heating a serum to 58° C. may increase its anaphylactic toxicity, and in a way my results support his hypothesis.

EXPERIMENTS WITH CASEIN.

Purifed caseinogen was found to be highly efficient in producing anaphylactic reactions, giving more fatal reactions than did milk in corresponding doses, as is shown by Table 5. Thus, 0.1–0.25 gm. purified caseinogen of cow milk usually produced fatal reactions in sensitized guinea-pigs, while with other animals sensitized in the same way 5–10 c.c. of milk, containing 0.15–0.30 gm. of

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[1] *Jour. Med. Res.*, 1910, 23, p. 6.

[2] *Centralbl. f. Bakt.*, 1909, 51, p. 576.

TABLE 5.
CASEIN.

	Sensitizing Dose	Days' Interval	Second Injection	Results	Subsequent Injections and Remarks
1....	0.025 gm. caseinogen, cow	21	0.25 gm. caseinogen, cow	Died in 15 min.	
2....	0.0025 " " "	21	0.25 " " "	" " 15 "	
3....	0.025 " " " (boiled)	17	0.16 " " "	" " 2 hrs.	The sensitizing caseinogen was heated 25 min. at 100°
4....	0.01 " " " "	17	0.16 " " "	" " 15 min.	" " " " " " " " "
5....	0.005 " " " "	17	0.16 " " "	Moderate symptoms	" " " " " " " " "
6....	0.01 " " "	21	10 c.c. cow's milk	" "	
7....	0.005 " " "	21	5 " " "	" "	
8....	0.025 " " "	17	10 " " "	Severe "	24 hrs. later no reaction to caseinogen
9....	5.0 c.c. cow's milk	17	0.16 gm. caseinogen, cow	Died in 35 min.	
10....	2.0 " " "	17	5.0 c.c. cow's milk	" after 2 hrs.	Died during night
11....	1.0 " " "	17	0.16 gm. caseinogen, cow	" in 2 hrs.	
12....	5.0 " " "	17	— goat casein	Doubtful symptoms	24 hrs. later reacted fatally to cow caseinogen
13....	0.01 gm. caseinogen, cow	26	— " "	Slight · "	(Dosage with goat casein doubtful because of poor solubility)
14....	0.01 " " "	21	" "	" "	
15....	0.002 " " "	21	" "	Severe "	24 hrs. later reacted severely to cow caseinogen
16....	0.001 " " "	21	" "	No "	
17....	0.0002 " " "	21	" "	Moderate "	24 " " " " " " "
18....	0.1 " goat casein	21	0.1 gm. cow caseinogen	Died in 65 min.	
19....	0.002 " " "	21	0.1 " " "	" " 50 "	
20....	0.001 " " "	21	0.1 " " "	Moderate symptoms	24 " " " slightly " goat casein
21....	0.0002 " " "	21	0.1 " " "	" "	24 " " " " " " "
22....	0.01 " " "	26	0.1 " " "	Severe "	
23....	0.01 " " "	26	0.1 " " "	" "	

caseinogen, seldom produced fatal reactions. Heating of caseinogen to 100° for 25 minutes did not materially impair its efficiency in producing fatal anaphylactic sensitization (experiments 11, 12, 13), a result to be expected since it has already been shown,[1] and repeatedly confirmed, that heating of whole milk does not destroy its power of causing anaphylactic sensitization or intoxication.

A suggestive result is shown in experiments 16–24, which indicate that caseinogen from the goat and cow interact with one another. This agrees with Bordet's[2] observation that guinea-pigs sensitized to cow milk react with goat milk; also with Fleischer,[3] who studied precipitin reactions with purified casein from different species of animals, and obtained interreactions differing only in degree. Bauer[4] also found that by the means of the complement fixation reaction casein could be differentiated from lactalbumin of the same species much more readily than from casein of different but related species, the latter observation applying to cow and goat casein. Here we have a case in which chemically related proteins from different species show strong relationships by biological reactions, while naturally associated proteins in the same species, when of widely differing chemical nature, show very little relationship.

EXPERIMENTS WITH COMPOUND PROTEINS.

In view of the general interest in the biological reactions of the so-called nucleoproteins, a number of attempts were made to study the reactions of these proteins as well as of their components, but with practically no success. A large quantity of ripe cod testes was obtained from the Woods Hole station of the Bureau of Fisheries, through the courtesy of Superintendent E. F. Locke. From this was prepared a histone, the "Gadushistone," which has been studied by Kossel and Kutscher.[5] Also a sodium nucleinate was prepared, and from the sperm-free aqueous extract of the testicles a protein resembling albumin was separated by precipitation with ammonium sulphate, and repurified by repeated precipitation.

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[1] Wells, *Jour. Infect. Dis.*, 1908, 5, p. 449.

[2] *Ann. Inst. Pasteur*, 1909, 23, p. 166.

[3] *Roussky Wratsch*, 1908, 49; Abst. in *Centralbl. f. Pathol.*, 1909, 20, p. 308.

[4] "Ueber den Artcharakter der Milcheiweisskörper," *Berl. klin. Wchnschr.*, 1910, 47, p. 830.

[5] *Ztschr. Physiol. Chem.*, 1900, 31, p. 188.

The albumin behaved like ordinary serum albumin or egg albumin, producing typical and fatal anaphylactic reactions, and being specific when tried against mammalian sera.

The nucleinate did not produce any reactions when guinea-pigs were given small sensitizing and larger intoxicating doses (0.1 gm.) at a three weeks' interval; a result to be expected, since no protein is present in the preparation.

The histone was so toxic of itself that its anaphylactic properties could not be studied; guinea-pigs given 0.1 gm. of this preparation becoming severely ill in a few minutes and remaining so for hours, the symptoms being quite similar to those of anaphylactic shock. Normal animals were fully as much affected by this protein as were those which had been given sensitizing doses. The toxicity was apparently not at all reduced by heating the neutral solution for one-half hour at 56°.

These results are similar to those obtained by Taylor[1] with salmon sperm, which was found to be highly toxic to rabbits, while no cytolytic antibody was found by immunization of rabbits to the isolated protamine or to the nucleinic acid. McCrudden[2] also has found fish ovaries to contain very toxic substances causing death of rabbits in a few minutes after subcutaneous injections. This material, however, was found in the albumin fraction.

A preparation of nucleoprotein was made from dog livers according to the method described by Beebe. This preparation on hydrolysis yielded a large amount of purines, and resembled in all respects the compound proteins of this class. Injected into guinea-pigs in 0.1 gm. doses, dissolved in 0.1 per cent NaOH, it was found to be somewhat toxic, and normal guinea-pigs reacted if anything more strongly to the nucleoprotein than did previously injected animals. Heating to 56° for one-half hour did not greatly lessen the toxicity of this preparation. Guinea-pigs sensitized to this nucleoprotein preparation in 0.1 gm. doses gave moderate reactions with dog serum, but doses of one to two milligrams did not make guinea-pigs sensitive to dog serum. It seems probable that the numerous chemical manipulations, especially the repeated solution in alkali and precipitation with acid, may be responsible for the inefficiency of these preparations of nucleoprotein and histone, for it is known that the action of acids and alkalies rapidly impairs the activity of proteins in respect to anaphylaxis, as well as other biological reactions.[1]

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[1] *Jour. Biol. Chem.*, 1908, 5, p. 311.

[2] *Ibid.*, 1911, 9, p. 9.

Interest in compound proteins having been aroused by the nucleoprotein work, experiments were made with another sort of compound protein, a *mucin* prepared from the gastric mucosa of pigs, and kindly furnished me by Professor L. B. Mendel. This glycoprotein was found capable of producing anaphylactic intoxication in sensitized guinea-pigs, the reactions generally being of moderate severity, but one fatal reaction occurring in six experiments. Animals sensitized with pig serum or with an extract of pig muscle tissue did not react to mucin, and the mucin did not render them refractory to pig serum. Some slight reactions were obtained when muscle extract was injected into the guinea-pigs sensitized with the mucin preparation. As this reaction occurred only in this direction it is probably to be interpreted that some traces of muscle or blood proteins were present in the mucin preparation. In any event, we have here another case of a protein from an animal showing a well developed independent biological specificity against the serum and tissues of the same species of animals.

ACTION OF AUTOLYTIC AND TRYPTIC ENZYMES UPON ANAPHYLACTIC ANTIGENS.

Previous experiments having shown that digestive enzymes destroy the anaphylactic properties of proteins without altering their specificity, a few experiments were performed to test the influence of autolysis in this respect. A lot of human placentas were allowed to autolyze two years under toluene in five volumes of water, at room temperature. The toluene was removed from a portion of the supernatant fluid, which contained 10 mg. of coagulable protein per c.c., and this protein solution was used. It was found to cause typical reactions, often fatal, when injected into guinea-pigs sensitized with either human serum or with the autolysis fluid. Guinea-pigs sensitized with the autolysis fluid reacted severely or fatally to human serum, but not at all to dog serum or horse serum. Apparently, then, this prolonged autolysis, not having removed all the coagulable protein, has not destroyed the sensitizing or intoxicating properties of the proteins in the solution; neither has it destroyed their specificity.

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[1] See *Jour. Infect. Dis.*, 1909, 6, pp. 509–13.

In this connection may be mentioned a continuation of an experiment on the destruction of the sensitizing property of serum by trypsin. It was previously reported (article II) that a specimen of bovine serum which had been digested with commercial pancreatic powder (pig pancreas) for 314 days at 37°, with fresh pancreatic powder added at intervals, still retained the power of sensitizing guinea-pigs to bovine serum when doses of 1 c.c. or more were used, but no anaphylactic intoxication could be demonstrated with 10 c.c. doses of the digestion mixture injected into sensitized guinea-pigs. The digestion of this same preparation of bovine serum has been continued, a small amount of pancreatin being added every few months. At the end of the second year of digestion the smallest amount of digestion mixture sensitizing an animal was 6 c.c., and at the end of three years only a slight reaction could be obtained when 10 c.c. sensitizing doses were used, but none at all with 5 and 7.5 c.c. sensitizing doses. Therefore three years' digestion has not entirely extinguished the power of this serum to sensitize guinea-pigs, although the degree of activity is extremely slight, when it is considered that fresh serum sensitizes in doses of 0.0001 c.c. to 0.00001 c.c.

SUMMARY.

Ovovitellin from the yolk of hen eggs when tested by the anaphylaxis reaction was found to be entirely distinct from crystallized egg albumin, and from ovomucoid from hen egg white, and also from vitellin from the yolk of turtle eggs.

Ovomucoid produces characteristic and specific anaphylaxis reactions, in spite of protracted boiling and repeated precipitation with alcohol. It is entirely distinct, according to this reaction, from crystallized egg albumin, from repurified "globulin" of egg white, and from ovovitellin, all from hen eggs.

The "globulin" fraction of hen egg white has about the same degree of intoxicating and sensitizing power as crystallized egg albumin; if anything it is slightly less active. In spite of the most careful separation of these two portions of egg white by means of ammonium sulphate precipitation, the resulting preparations each react almost as well against the other as against itself.

Guinea-pigs sensitized with several antigens can be saturated with one of these antigens and then will react severely to one of the others. By this means it can be shown that there are in the "globulin" fraction and in the crystallized albumin, specific and distinct antigens, and also a common antigen which cannot be separated from them by fractional precipitation with ammonium sulphate.

Therefore it is demonstrated that in hen egg white there are at least four antigens (three in the coagulable protein and one non-coagulable) which can be distinguished by the anaphylaxis reaction, and therefore are biologically distinct from one another although coming from a single secretion. At least one other protein of the egg, the ovovitellin, can be differentiated from the other three egg antigens, so that we have here demonstrated five antigens in the egg which are biologically distinct in spite of a common origin. These must be looked upon as examples of chemical specificity independent of species specificity. The antigens distinguished by the anaphylaxis reaction seem to correspond to the proteins which have been distinguished by chemical means.

Guinea-pigs fed upon a certain protein are at first rendered sensitive to this protein. After some time, however, if the feeding is continued they become less sensitive, until they reach an immune or refractory condition so that they do not react to two spaced injections of the fed protein. This refractory condition seems to be reached more easily with the vegetable proteins of the natural food (corn, oats) than with animal proteins.

Crude egg white has less sensitizing and intoxicating power than corresponds to the activity of the isolated proteins from equal amounts of egg white. It has not been possible to determine the nature of the inhibiting substance which accounts for this depression of the activity of the proteins of crude egg white.

Pure caseinogen gives strong anaphylaxis reactions, and the casein of cow milk and of goat milk react freely against each other.

Sodium nucleinate from cod sperm does not give anaphylaxis reactions. Albumin from cod sperm gives typical and specific anaphylaxis reactions. Histone from cod sperm is toxic to guinea-pigs, retaining its toxicity when heated to 56°, and not producing demonstrable anaphylaxis reactions.

Mucin from the pig's stomach produces typical anaphylaxis reactions, and is specific against pig's blood serum or extracts of pig muscle.

Autolysis of two years' duration, but without destruction of all coagulable proteins, did not destroy the anaphylactic antigen of human placenta extract, and did not destroy its specificity. Three years' digestion of bovine serum with trypsin almost but not quite destroyed its sensitizing power, the minimum sensitizing dose being 10 c.c.; at the end of two years' digestion good reactions were obtained when the sensitizing dose was 6 c.c.